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AN INVESTIGATION OF THE EXTENT OF THE BACTERIAL POLLUTION OF THE ATMOSPHERE BY MOUTH SPRAY.*

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INTRODUCTION.

WITH the progress of exact knowledge sanitarians have been led to place less and less emphasis upon the part played by the atmosphere in the spread of zymotic disease. Bacteriological studies have shown that quietly expired air is germ free and epidemiological investigations indicate that little place is left for aerial transfer of disease in any form (Chapin, 1908). Even sewer air has been shown by recent work to be on the whole remarkably free from pollution with sewage bacteria.

On the other hand, Horrocks and Andrewes in England have demonstrated that a local pollution of sewer air does occur in the presence of mechanical splashing, taking the form of a fine spray of sewage, temporarily suspended in the air. Similarly, the work of Flügge and his pupils has shown that in sneezing, coughing, and loud speaking a spray is thrown out which contaminates the air for a considerable distance with bacteria from the mouth. It has been generally assumed that this mouth spray may be an important factor in the spread of tuberculosis, and other diseases affecting the respiratory tract.

The real importance of the mouth spray, as of any other vehicle of disease, can be properly determined only by quantitative investigations. The day of qualitative sanitation is fast passing. Not, "Is such a thing dangerous?" but, "How dangerous is it?" is the question we ought to ask. Applying this criterion to the bacterial content of sewer gas one of the writers has been led to the conclusion that on the whole the danger of infection from that medium is very slight (Winslow, 1909). In the present investigation the attempt has been made to apply similar quantitative standards to the mouth spray,

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and to determine whether the extent of air pollution with mouth bacteria under normal conditions is sufficient to be measured bacteriologically and to be estimated as a practical factor in the spread of zymotic disease.

PREVIOUS INVESTIGATIONS ON THE DISCHARGE OF BACTERIA IN MOUTH SPRAY.

It is particularly to Flügge and his pupils that we owe the most careful studies of the possibilities of aerial transmission of disease germs, either in the form of dust or in the form of fine spray (Flügge, 1897*a* and 1897*b*). He was the first to lay special stress upon the danger from the spray produced in coughing in the case of tuberculosis (Flügge, 1899), and to point out how much more important this danger is than the

TABLE 1.
DISTRIBUTION OF BACTERIA BY MODERATELY LOUD SPEAKING.
Laschtschenko (1899).
Room, 90 cu.m. Speaking for 1 hour.

Distance of plate from speaker. Meters.....	0.5	0.75	1.0	1.25	1.5	1.75
Total plates exposed.....	1	3	1	2	3	2
Negative plates.....	0	0	0	0	0	0
Mean number <i>B. prodigiosus</i> per plate.....	450	32	10	6	4	8

TABLE 2.
DISTRIBUTION OF BACTERIA BY LOUD SPEAKING (ONE HOUR).
Laschtschenko (1899).

Distance. Meters	Mean Number <i>B. prodigiosus</i> per Plate					
	1	2	3	4	5	6
Small room (50 cu.m.)	1	3	2	0	1	1
Large room (90 cu.m.)	5	10	..	3	..	..

possibility that the disease may spread through the blowing about of dried sputum which before this time had been accepted on the basis of Cornet's classic work (Cornet, 1889) as the principal mode of disseminating the disease.

The first important experiments upon mouth spray were carried out by Laschtschenko, and upon them Flügge's earlier papers were largely based, altho the full results were not published until two years later (Laschtschenko, 1899). The general method employed in these experiments consisted in inoculating the mouth with a heavy suspension of *B. prodigiosus* in normal salt solution and then causing the subject to cough, sneeze, or speak loudly for a specified length of time. Agar plates were exposed at various distances to receive any bacteria which might settle upon them. Some of the experiments were made in a glass chamber of 3.2 cubic meters capacity provided with an opening for the mouth of the investigator; others were carried out in rooms of 50 and 90 cubic meters capacity.

Quiet speaking, in the glass chamber, showed a few colonies; in the larger room, none. The results for moderately loud speaking and for loud speaking we have tabulated for convenient reference in Tables 1 and 2.

A general summary of all the loud and moderately loud speaking experiments grouped together is given in Table 3.

Coughing and sneezing of course gave higher results which need not be considered in detail. Sneezing distributed the infection for nine meters.

The most significant of Laschtschenko's experiments were carried out with tuberculous patients. The coughing of such a patient while sitting for an hour in the glass chamber, to which reference has been made, distributed virulent tubercle bacilli, as evidenced by animal tests made with normal salt solution exposed in the chamber during the test. The actual extent of the air pollution was however slight, as shown by drawing off the air of the chamber, filtering, and injecting into guinea-pigs. When less than 2 cu.m. of air per hour were examined, no positive results were obtained; but out of five tests covering a period of five hours each, and involving the filtering of 10 cu.m. of air in each case, two tests showed virulent tubercle bacilli.

Hübener (1898) carried out a series of similar experiments with a view to the danger of infecting patients upon the operating table from the mouth. His method consisted in exposing four agar plates about 50 cm. in front of and below the face of a subject who had infected his mouth with *B. prodigiosus*. In 11 experiments in which the subject counted aloud for 10 minutes the number of colonies on the four plates

TABLE 3.
GENERAL SUMMARY OF LASCHTSCHENKO'S EXPERIMENTS.

	90 cu.m. Room	50 cu.m. Room
Total plates.....	24	12
Mean number <i>B. prodigiosus</i> per plate.....	25	1.5
Per cent of negative plates.....	8	33

varied from 101 to 1,507 and averaged 458; two tests with three or four coughs gave 223 and 265 colonies and in two tests a single sneeze gave an uncountable number.

Along similar lines, Hamilton (1905) has shown that streptococci are expelled from the mouth in coughing, speaking, and even forcible breathing, and has emphasized the danger of surgical infection from this source.

V. Weismayr (1898) obtained somewhat different results which indicated a more restricted distribution of mouth spray than that reported by other observers. He found *B. prodigiosus* on plates directly in front of a coughing subject in quiet air up to a distance of 4 meters, but not beyond and not out of the zone directly in front of the mouth. When the air of the room was agitated, however, he found small numbers of bacilli 1-2 meters behind and at the side of the subject. In speaking experiments infection could not be traced at distances over 1 meter. Slides exposed before the mouths of coughing consumptives showed tubercle bacilli only rarely.

The most exhaustive of all the experimental studies of the spread of *B. prodigiosus* in mouth spray was perhaps that carried out by Koeniger (1900). He followed in the main the methods of Laschtschenko, making in all 18 speaking experiments in two rooms, one of 97, the other of 440 cu.m. capacity. His main results have been summarized in Table 4.

Koeniger observed also the amount of infected spray discharged by enunciating various letters and showed that consonants like *p* and *t* caused the greatest pollution. The amount of spray of course increased with loudness and sharpness of speech but, as indicated in the table below, whispering, with its clear enunciation, gave higher results than gentle speech in an ordinary tone.

TABLE 4.
DISTRIBUTION OF BACTERIA IN MOUTH SPRAY.
(Koeniger, 1900.)
Speaking, generally for 15 minutes.

	TONE OF VOICE				
	Sharp	Loud	Moderately Loud	Soft	Whisper
Total plates.....	30	101	143	23	24
Mean number <i>B. prodigiosus</i> per plate.....	14.5	7.8	2.1	1.4	5.3
Total negative plates.....	0	9	91	16	10
Per cent negative plates.....	0	8.9	63.6	69.6	41.7

Koeniger found *B. prodigiosus* on his plates in all parts of the room, at extreme distances of 12.4 meters in front of the speaker and at one side of and behind him. In this respect his results differ from those of v. Weismayr. On the other hand, he made important observations on the length of time for which the bacilli would remain in the air which contradict Flügge's conclusion, based on experiments with atomizer spray, that bacteria from the mouth might remain suspended in the air for 4 or 5 hrs. Koeniger's general results are brought together in the table below. *B. prodigiosus* was never found in quiet air an hour after the speaking had ceased, tho when the air of the room was agitated by moving about and opening and shutting doors, positive results were obtained after an hour and a half.

TABLE 5.
SETTLING OUT OF MOUTH SPRAY IN QUIET AIR.
(Koeniger, 1900.)

Interval between Speaking and Exposure of Plates. Minutes	Percentage of Colonies Found, Taking Number on Plates Exposed during Speaking as 100
0	95
10	38
20	9.8
30	5.5
45	2.7
60	0.7
90	0.0
105	9.6*
120	0.0
150	0.0

* Air agitated.

Experiments with *B. mycoides* made in similar fashion showed a much more limited distribution of mouth spray than was observed in the case of *B. prodigiosus*. Out of 105 plates exposed during 15 minutes' speaking only 23 showed colonies of the specific germ. The average number of colonies per plate was 7 and the maximum distance 3 meters. All plates exposed 10 minutes after speaking were sterile.

Gordon in England was the next to take up this work and his investigations confirmed in all respects the work of Laschtschenko and Koeniger (Gordon, 1904). Repeating the *B. prodigiosus* experiments he traced aerial infection for 12.2 meters in front of the subject and for 3.7 meters behind him. We have summarized these particular experiments in Table 6 below. Gordon's most important contribution to the whole subject lay in the suggestion that the mouth streptococcus might be used as a normal

index of mouth pollution without the use of artificial *B. prodigiosus* inoculations; but this aspect of his work will be considered farther on.

Mention may perhaps be made here of experiments by Flügge (1897), Buchner, Megele, and Rapp (1899), and Hutchison (1901), which have shown, as might be expected, that bacteria artificially sprayed into the air can be transported by air currents of low velocity for considerable distances. Buchner, Megele, and Rapp found a limiting velocity of 1 mm. per second for *B. prodigiosus* and 1.3-1.8 mm. per second for yeast cells 3-10 microns in diameter. Little can be learned from such experiments as to the behavior of the mouth spray itself which may be in a much grosser state of division.

So far reference has been made for the most part only to experiments in which *B. prodigiosus* was used as an indicator of the distribution of bacteria in the mouth spray. Another important series of investigations has dealt more directly with the practical

TABLE 6.
DISTRIBUTION OF BACTERIA IN MOUTH SPRAY.
(Gordon, 1904.)
Generally, loud speaking for 1 hour.

Total plates exposed.....	30
Mean number <i>B. prodigiosus</i> per plate.....	0.7*
Total negative plates.....	14
Per cent negative plates.....	47

* Number on four positive plates not stated.

question of the spread of tuberculosis by observations of the behavior of the tubercle bacillus itself. Laschtschenko's work along this line has already been discussed. At about the same time Englemann (1898) and Heymann (1899) showed that tubercle bacilli could be detected on plates of various sorts exposed within 0.5-1 meter of coughing consumptives; and Heymann succeeded in infecting 6 out of 25 guinea-pigs exposed 20-45 cm. in front of coughing consumptives, with heads fixed toward the patients.

Fränkel (1899) found that out of 219 masks worn by tuberculous patients 52 showed macroscopic evidence of contamination with sputum; in 26 of the latter tubercle bacilli could be demonstrated by staining. Fourteen patients wore the masks in these experiments. Two yielded positive results almost always, one frequently, 3 once each, and 8 never showed tubercle bacilli on the masks.

In a later investigation, Heymann (1901) shut up a coughing consumptive patient in a small chamber for an hour or two. At the close of this period the patient left the chamber and broth plates were exposed, after the chamber had remained quiet, for varying periods, 15, 30, or 90 minutes. Of 24 guinea-pigs inoculated with broth thus exposed, two which had received broth exposed 30 minutes after the end of the coughing developed tuberculosis. In a second series of tests the air in the chamber was drawn off and washed, and the sediment in the washings centrifuged and injected. Of 14 guinea-pigs injected with the sediment from 7 cu.m. of air drawn off while the coughing was in progress only two became infected; of 22 guinea-pigs injected with the sediment from 11 cu.m. of air drawn off at periods of 15-60 minutes after the coughing stopped, all remained sound. Many more recent observers have shown that tubercle bacilli may be found on plates exposed before coughing consumptives. The most exhaustive of these investigations is perhaps that of Ziesché (1907). He arranged a

vertical plate having an area of 324 square centimeters at a distance of 40–80 cm. from the mouth of phthisical patients and counted under the microscope the number of tubercle bacilli discharged in half an hour. Of the patients examined only once 12.5 per cent gave positive results; of those frequently examined, 78.9 per cent. Altogether 29 out of 62 tests were positive. Of the 29 positive plates, 3 showed less than 10 bacilli, 15 from 10 to 100, 7 from 100 to 500, 2 from 500 to 1,000, one 1,445, and one 20,174 bacilli.

Similar observations have been made in the case of leprosy. Schäffer (1898) found leprosy bacilli on glass plates exposed before the mouth of a leper after 10 minutes' reading. Plates near the mouth showed many thousands of bacilli and infection could be traced to a distance of 1.5 meters. These experiments were repeated and confirmed by Hübener (1898) in a paper to which reference has been made above.

It is sufficiently clear from a consideration of all these investigations that bacteria may indeed be discharged from the mouth in such a way as to spread disease. On the other hand it seems equally clear from the tuberculosis experiments that the practical danger is confined to a local discharge of spray rather close to the mouth of the patient. Rightly interpreted there is no basis in any of this work for the conclusion that disease is spread broadcast through the atmosphere. Professor Flügge's own treatment of the matter is judicial and conservative. He lays stress only on coughing, not on speaking, as a possible danger. He points out that in experiments carried out by his pupils tubercle bacilli were abundant only within 0.5 meter of the coughing patient and that beyond 1.5 meters their number was so small as to make the chance of infection practically nil. He notes that only certain phthisical patients discharge infected spray when they cough and that others do so only at certain periods of the disease, and only at certain times of day. In one of his first papers (Flügge, 1897*b*), he says: "Altogether it appears that under natural conditions the infection resulting from sputum spray is not so significant as one might think at first sight." In a later paper (Flügge, 1901) the following rational rule is laid down: "During strong paroxysms of coughing, the consumptive should keep at arm's length from his companions and should hold a handkerchief before his mouth. In workrooms, offices, and such places the space between the heads of the workers should be at least 1 meter."

No exception can be taken to such conclusions as these. Unfortunately, however, others have not been so conservative. The B. prodigiosus experiments particularly have lent themselves to mis-

leading interpretations. It is common to see statements to the effect that bacteria are discharged from the mouth in coughing, sneezing, and even loud speaking to distances of 12 meters and in all directions behind as well as in front of the subject; and from such unqualified statements the inference is implicitly drawn that a general infection of the atmosphere takes place over a wide radius and that there is real danger of infection from breathing the air in the neighborhood of a consumptive. Fränkel (1899) early took up an extreme position, speaking of spread through the air as the "most frequent cause of tuberculosis," and recommended the wearing of masks by tuberculosis patients discharging bacilli freely. This was ten years ago, it is true; but Tendeloo (1908) at the last Tuberculosis Congress said: "It has been proved by numerous experiments (Cornet, Flügge, etc.), that bacteria floating in the air, either in dry dust particles, or suspended in minute fluid particles, may be inhaled into the bronchi, bronchioli, and air-vesicles, where they fall down just like inhaled dust particles;" Pannwitz (1908) said: "If they contain a coughing tuberculous patient, inclosed spaces, such as dwelling-houses, workshops, counting-rooms, public halls, railway coaches, and steamer cabins, are speedily converted into tuberculosis-inhalatoriums in which healthy persons may infect themselves;" and Bernheim (1908) recommended the wholesale disinfection of air on the ground that "there is an intimate relation between tuberculosis and the microbes of the air. The more highly the atmosphere is charged with bacteria the greater is the number of consumptives living in that unhealthy environment."

As a matter of fact it can be shown that even the *B. prodigiosus* experiments do not indicate any general atmospheric infection in the proper meaning of the term; and studies of the discharge of tubercle bacilli and of normal mouth bacteria show that the spread of these organisms is insignificant in comparison with the discharge of *B. prodigiosus* in culture media with which the mouth has been artificially infected.

REPETITION OF EUROPEAN EXPERIMENTS ON AIR POLLUTION BY MOUTH SPRAY USING *B. PRODIGIOSUS* AS A TEST ORGANISM.

The first object of our own experiments was to extend and place on a quantitative basis the work of Flügge's pupils on air pollution

by mouth spray. It was necessary therefore to follow their procedure rather closely, using *B. prodigiosus* as a test organism and detecting it by the simple exposure of plates, in order to be sure that the conditions of their work were fairly duplicated. At the same time we examined measured volumes of air and determined the actual number of specific germs present by methods discussed in the succeeding section.

Three methods were employed in our experiments for inoculating the mouth with *B. prodigiosus*. The first was to pour water on an agar slant culture, shake so as to get a suspension, rinse out the mouth with the suspension for a minute, and reject the surplus water. The second was to transfer the organisms directly from the culture to the mouth by means of a platinum loop. The third was to transfer the bacteria on a loop to a small quantity of water (1 or 2 c.c.) which could be entirely retained in the mouth. The last method proved most satisfactory. Analyses of the saliva at the close of the experiments generally showed that *B. prodigiosus* was present in 10^{-6} c.c.

In the first 10 experiments the subject read from a book in a loud tone and at a fairly rapid rate. Beginning with Experiment 11 and continuing through the series the subject repeated from memory verses in English and German, in a loud tone and with exaggerated enunciation. The discarding of the book appeared to facilitate the spread of bacteria.

The tests were carried out in three laboratories of the Institute of Technology. Two of them are about 6×8 meters in area and 3.7 meters high; the third is 11×8 meters and 3.7 high. In the qualitative work ordinary plates were exposed in different parts of the rooms at varying heights and at varying distances from the speaker. The speaking usually lasted for 15 minutes and the plates were exposed during this time and for a varying period, generally three-quarters of an hour afterward. The speaking was done by four different subjects during the course of the experiments.

The general results obtained by the simple exposure of Petri plates are indicated in Table 7. The speaking was continued for 15 minutes in each case except in Experiments 14 (7 minutes), 15 (13 minutes), and 21 and 23 (10 minutes).

It will be noticed that the substitution of speaking for reading,

TABLE 7.
B. PRODIGIOSUS ON PLATES EXPOSED IN ROOM POLLUTED BY MOUTH SPRAY.

EXPERIMENT	PERIOD OF EXPOSURE MINUTES	NUMBER OF PLATES	TOTAL COLONIES	MEAN PER PLATE	NEGATIVE PLATES	
					Total Number	Percent-age
7.....	15	11	1	.09	10	91
8.....	15	12	2	.16	11	92
9.....	15	18	2	.11	16	89
10.....	15	21	4	.19	17	81
11.....	75	25	22	.88	14	56
12.....	15-75	42	3	.07	39	93
13.....	75	24	22	.92	10	42
14.....	..	25	9	.36	18	72
15.....	73	25	20	.80	16	64
16.....	135	13	34	2.6	3	23
17.....	..	11	68	6.2	3	27
18.....	45	10	134	13.4	0	0
19.....	60	12	88	7.3	0	0
20.....	60	14	75	5.4	0	0
21.....	60	14	142	10.1	0	0
22.....	75	12	56	4.7	4	33
23.....	25-70	36	98	2.7	8	22
Totals and averages.....		325	780	2.4	169	52

at and after Experiment 11, caused an increase in positive results and that as the later experiments progressed the number of bacteria on the plates continued to increase, perhaps as a result of more thorough inoculation of the mouth or more vigorous enunciation. Control plates, 22 in number, exposed just before Experiments 19 and 20, were all negative, showing that no serious permanent pollution of the room air had taken place.

In general these results agree closely with those obtained by the German and English observers cited earlier in the paper. The more important data are presented for comparison in Table 8.

TABLE 8.
COMPARATIVE RESULTS OF VARIOUS OBSERVERS ON EXPOSURE OF PLATES IN ROOMS POLLUTED BY MOUTH SPRAY.

Observer	Total Plates Exposed	B. prodigiosus. Mean Colonies per Plate	Percentage of Negative Plates
Laschtschenko.....	36	17.2	17
Koeniger.....	321	5.2	39
Gordon.....	30	0.7	47
Winslow and Robinson.....	325	2.4	52

Laschtschenko's figures are a little high; but with that exception the results are entirely concordant. It is clear that loud speaking discharges bacteria from the mouth in the form of spray; and that by inoculating the mouth of the speaker with *B. prodigiosus* and

exposing plates in the room the specific germ may be detected in considerable numbers.

Suggestive data in regard to the distribution of the mouth spray in space may be obtained by analyzing the results of our experiments according to the distance of the plates from the position of the speaker. The results for each point at which ten or more plates were exposed in different experiments are brought together in Table 9. The heaviest pollution appears to be between 2 meters and 4.5 meters from the speaker. The spray from the mouth probably shoots over areas which are too near; and the particles which are carried beyond 4.5 meters are fewer in number.

TABLE 9.
B. PRODIGIOSUS ON PLATES EXPOSED AT VARIOUS DISTANCES FROM THE SPEAKER.

Distance Meters	Total Plates	Total Colonies	Colonies per Plate
1.0.....	13	2	.15
1.5.....	28	62	2.22
2.0.....	48	199	3.96
2.5.....	22	126	5.73
3.5.....	50	208	4.16
4.5.....	13	40	3.08
5.5.....	33	61	1.85
7.0.....	14	32	2.28
7.5.....	31	42	1.35

Some idea of the general extent of superficial pollution due to mouth spray may be gained from the following considerations. In the room used for all the plate experiments except 7, 9, and 12, 254 plates were exposed and 774 colonies of *B. prodigiosus* developed, an average of 3.5 colonies per plate. The total area of the room was 480,000 sq.c. and the mean area of a plate 54.5 sq.c. Assuming that all the organisms above a plate settled upon it, the mean number of *B. prodigiosus* in the whole room in each experiment would be $\frac{480,000 \times 3.5}{54.5}$ or 30,830, and the number discharged on a square meter of surface (by 15 minutes' speaking) would be 646.

QUANTITATIVE METHODS OF ENUMERATING BACTERIA IN THE AIR.

The various processes suggested for enumerating bacteria in the air may be classified broadly under two main heads, filtration methods and sedimentation methods. In one case a measured volume of air is filtered through a powdered solid medium (asbestos, salt, sugar, sodium sulphate, cotton, sand, glass wool) or is bubbled through a

liquid. The solid filtering medium is washed in sterile water and aliquot portions of the water are plated; or portions of the liquid through which the air has been bubbled are plated directly. In all these procedures there is some danger that bacteria may be lost if the medium is not of sufficient fineness, or if the passage of the air is too rapid or if the connections of the apparatus are not all tight. The proper collection of samples with a fine filter on the other hand is time consuming.

The second group of methods involves the exposure of surfaces of nutrient media upon which the bacteria from a known volume of air may settle out and on incubation develop colonies. In its most primitive form ordinary Petri plates are left open in a room for known periods of time. The objection to this is twofold. In the first place the bacteria which fall upon the plates are not related to any determinable quantity of air. In the second place their number will vary greatly with the effect of chance air currents. Hesse long ago founded a more exact method upon this general principle by drawing air slowly through a long cylindrical tube lined with nutrient medium. Recently an improvement on this procedure has been suggested by one of us (Winslow, 1908) involving the use of two liter-and-a-half bottles, having gelatin on the bottom. The bottles are joined by tubing in tandem and a liter of air is drawn in by another water-suction bottle and the bacteria allowed to settle out. The chief objection to this procedure lies in the cumbrousness of the apparatus which requires the carrying about of two large bottles for every sample to be examined. For investigations in the laboratory, however, this procedure has many advantages.

In the present study three methods have been used. Most of the determinations were made by the culture bottle method; and in connection with this procedure two methods of collecting samples have been tried, one the usual method of drawing in air by connecting the second culture bottle with a water-aspirator, the other a vacuum method like that used in collecting air samples for carbon dioxide. In this case the culture bottle is first prepared by covering the bottom with gelatin, plugging with cotton, and sterilizing. At the same time a number of vaselined one-hole rubber stoppers plugged with three-inch lengths of capillary tubing are separately sterilized

in a pipette case. If the bottles were sterilized with the rubber stoppers in them the breakage would be heavy; but after sterilization the stoppers may be substituted for the cotton plugs. Each bottle is then attached by the capillary tube to a suction pump and the air is exhausted for about 10 minutes or until a 90-95 per cent vacuum has been obtained. The capillary tube is now sealed in a flame and the sample of air may be taken at any time by removing the stopper. The danger of leakage from imperfectly fitting stoppers is considerable in this method and many bottles must be discarded but there is a great saving of time in sampling.

Another method used in a few determinations is a simple modification of the plate method designed to secure quantitative results with protection from air currents. An ordinary plate is opened and a covered cylinder a little larger than the plate and about 20 cm. high is placed over it. The apparatus is allowed to stand for half an hour so that the bacteria may settle. Finally in some of the later experiments with mouth bacteria the sand filter method was used, air being drawn by means of a suction bottle through a tandem filter, each member holding a 1.5 cm. layer of sand passing a sieve with 100 meshes to the inch.

QUANTITATIVE STUDY OF AIR POLLUTION BY MOUTH SPRAY USING
B. PRODIGIOSUS AS A TEST ORGANISM.

There are two objections to the method of exposing Petri plates as a measure of true air pollution. In the first place the procedure is necessarily an inaccurate one since the number of bacteria settling is conditioned not only by the number present in the air but also by the amount of motion in the atmosphere. In a strong current of air the plate method must yield results which are relatively too low. On the other hand in quiet air with heavy particles falling through it the results of the plate method must be too high. In the special case with which we are concerned the loud speaking, coughing, or sneezing produces a fine rain which falls more or less rapidly during the period of actual speaking and for a short time afterward. The rate of fall for small falling bodies may be approximately determined from Stoke's law which for spheres of density 1, calling the density of air .00018 and calling g 980, reduces to the form

$$v = 1,200,000r^2$$

where v is in centimeters per second and r is the radius of the falling particle in centimeters. An ordinary bacterium with a radius of about 0.0001 cm. should therefore fall through quiet air at a rate a little under 0.012 cm. per second, or about 17 inches per hour. If single isolated bacteria were discharged in the mouth spray their fall would be slow and would go on for several hours. As a matter of fact however such is not the case, as is made clear by Koeniger's experiments which have been cited above. He found that 60 per cent of the bacteria in the mouth spray disappeared from the air in 10 minutes, while after 20 minutes less than 10 per cent of the original number remained.

In two of our experiments similar data were obtained by opening all the plates before the speaking but removing some of them after various intervals. The results as shown below indicate no increase in bacteria after the actual period of speaking itself.

TABLE 10.
DURATION OF AIR POLLUTION BY MOUTH SPRAY.
Total *B. prodigiosus* on plates.

Period of exposure after speaking. Minutes.....	0	15	30	60
Experiment 12.....	1	2	..	0
Experiment 23.....	..	36	34	28

Clearly the mouth spray is a fairly coarse rain which settles out for the most part in 15 or 20 minutes. With such a rain as this the exposure of plates must give an exaggerated idea of the bacterial content of the air. Everything from the air above falls on the plates; but a liter of air should be credited only with the bacteria which it contains at a given moment. Many, even of these, cannot fairly be considered as constituting real aerial pollution if they are in large droplets, for such droplets would be too heavy to be drawn into the mouth with the inspired air.

In order to distinguish between coarse rain falling through the air and finer mist suspended in it, for a period of some minutes at least, we made quantitative determinations by the culture bottle method in Experiments 2, 5, and 6 and in Experiments 15-23, and by the covered plate method in Experiments 7, 8, 22, and 23. In the first three experiments the samples were collected during the speaking by another person. In the last nine the samples were collected just after the

speaking by another person, or by the speaker himself with carefully washed hands. The results of the culture bottle tests are shown in Table 11 below.

TABLE 11.
QUANTITATIVE POLLUTION OF ATMOSPHERE BY MOUTH SPRAY.

Experiment	Duration of Speaking, Minutes	Mean Colonies per Plate Exposed at Same Time	Number of Liter Samples	Number of Samples Showing <i>B. prodigiosus</i>
2.....	50	..	5	0
5.....	15	..	3	0
6.....	35	..	9	0
15.....	13	0.8	8	0
16.....	15	2.6	5	0
17.....	15	6.2	3	0
18.....	15	13.4	11	1
19.....	15	7.3	8	0
20.....	15	5.4	8	0
21.....	10	10.1	10	2
22.....	15	4.7	16	0
23.....	10	2.4	19	0
Total.....			105	3

Examinations were made by the covered plate method described above in Experiments 7, 8, 22, and 23. Immediately after the close of the speaking the plates were opened and covered with the cylinders which were allowed to stand 30 minutes for the bacteria present to settle. This period is theoretically too short but as a matter of fact the results as shown in Table 12 below were higher than those obtained by the culture bottle method.

TABLE 12.
AIR POLLUTION BY MOUTH SPRAY MEASURED BY COVERED PLATE METHOD.

Experiment	Duration of Reading, Minutes	Mean Colonies per Plate Exposed at Same Time	Number of Samples	Mean Volume of Air Inclosed	<i>B. prodigiosus</i> Found
7.....	15	0.09	10	840	0
8.....	15	0.16	11	840	0
22.....	15	4.7	7	1,370	2
23.....	10	2.4	7	1,370	2
Totals and averages			35	1,050	4

Altogether taking both quantitative methods into account 140 liters of air were examined and *B. prodigiosus* was found seven times. The four positive tests with the covered plate were obtained at points about 2 meters in front of the speaker. Two of the positive culture bottles were at the extreme end of the room, nearly 7 meters from the speaker; the other was at a distance of more than 5 meters. These

last results were obtained in the two experiments out of the whole series (see Table 11) which gave the largest number of colonies on the plates.

GORDON'S SPECIFIC TEST FOR AIR POLLUTION.

There is one weak point in all experiments involving an artificial inoculation of the mouth. We have no certainty that the bacterial emulsion introduced in this way behaves as the normal or pathological fluids of the mouth would do. It seems highly probable that a liquid with which the mouth has but just been rinsed would be ejected more freely than the sputum itself. In fact Heymann (1899) and Ziesché (1907) were able to show that in the mouth spray of a consumptive there are several distinct types of droplets which can be distinguished under the microscope and that it is only in droplets derived directly from the bronchi with but little admixture of mouth saliva that the tubercle bacilli are abundantly present. Some more direct test of normal mouth pollution than that furnished by the *B. prodigiosus* experiments is therefore greatly to be desired.

The real desideratum in the bacterial examination of air is a test which shall quantitatively detect the presence of some normal mouth bacterium which might serve as an index of mouth pollution as the colon bacillus serves in water analysis as an index of intestinal pollution. Such an index has been suggested by Gordon (1904), but its value has not been confirmed by subsequent investigations so far as we are aware. Gordon pointed out that one of the commonest organisms in the saliva is a streptococcus which he called *Strept. brevis* and which has since been more fully described by Andrewes and Horder (1906) as *Strept. salivarius*. It is a short chained form which renders broth uniformly turbid, clots milk, reduces neutral red, and ferments saccharose, lactose, and raffinose. Gordon found streptococci capable of fermenting lactose present in from one-millionth to one-hundred-millionth of a cubic centimeter of saliva. Similar streptococci which fail to ferment lactose or ferment neutral red were found to be present in ordinary street air but lactose-fermenting streptococci were absent. Gordon examined 10 samples of 50 liters of street air for streptococci by bubbling it through salt solution which was later mixt with nutrient broth. He found a lactose-fermenting streptococcus only once.

On the other hand, he found that the mouth streptococci could be easily detected in rooms polluted by mouth spray. His method consisted in exposing plates 3.5-5 inches in diameter containing neutral red broth, which was later incubated under anaerobic conditions. In a small room, 4.7 by 4.0 meters in area, 2 out of 6 plates showed mouth streptococci after 15 minutes' loud speaking, 4 out of 6 showed them after half an hour's speaking, and in 5 separate experiments after one hour of speaking 22 out of 30 plates gave positive results. In a larger room, 14.9 by 11.6 meters, 11 out of 34 plates and 28 out of 40 plates were positive after one hour's loud speaking. Quiet speaking and reading yielded negative results. These results coincide closely with those of the German experiments. Like them they measure the superficial discharge of spray but give no clear idea of the quantitative distribution of bacteria in the air above.

QUANTITATIVE STUDY OF AIR POLLUTION BY MOUTH SPRAY USING
THE PRESENCE OF MOUTH STREPTOCOCCI AS AN INDEX.

Our final experiments were devoted to the examination of air polluted by mouth spray with a view of determining to what extent the mouth streptococci can be detected in the air itself by ordinary quantitative methods.

In the first test of this sort the air was examined by the culture bottle method, lactose broth being used in the bottles instead of gelatin. The bottles were placed on a table directly in front of the speaker and from 0.6 to 2.4 meters away. In the first half of the work the speaker's mouth was 0.7 meters above the table and in the second half 1.2 meters above. Five liters of air were first examined for controls. Then the subject orated for 20 minutes, 4 liters of air being examined after each five-minute period. None of the 16 samples showed lactose-fermenting streptococci.

In the second and third experiments the air was examined by the sand filter method. In the second experiment, five different subjects orated loudly for a period of 10 minutes apiece (one taking part twice, so that six samples of air were examined). While the speaking by each subject was going on a sample of 4.5 liters of air was collected by attaching a water aspirator to a tandem sand filter

placed between 0.9 and 1.5 meters away from the speaker and directly in front of him. Each filter in the pair held a layer 1.5 cm. deep of sand which had passed a sieve having 100 meshes to the inch (finer than 0.25 mm.). After collecting the sample the sand was poured into bent dextrose broth tubes and incubated for four days at 37°. Litmus lactose agar plates were made from the broth tubes which showed growth. No lactose-fermenting streptococci were found in any case so that the 27 liters of air examined in this experiment were again all negative.

The third experiment was similar to the second, except that conditions were more severe. Seven tests were made, with two different subjects. The speaking was in English and German and was loud with very vigorous enunciation. In two tests the speaking was interspersed with considerable coughing. Samples of 4.5 liters each were collected during each period of speaking (10 minutes) from a point 35 cm. away from the speaker's mouth and 15 cm. below it. The sand was incubated in dextrose broth for a week, and litmus-lactose plates were inoculated at intervals from the broth tubes. No lactose-fermenting streptococci were found in this examination of 31 liters of air.

Altogether 74 liters of air exposed to pollution by mouth spray were examined by these quantitative methods for mouth streptococci with uniformly negative results. From these experiments it appears, as might be expected, that the pollution of the atmosphere by normal mouth streptococci is considerably less than the contamination with *B. prodigiosus* after inoculating the mouth with that organism.

Gordon's experiments, made with plates exposed during an hour's reading, of course constituted a very severe test since the plates received all the heavy spray which fell through the air during that period. We made a final experiment along similar lines to his, using litmus lactose agar instead of broth. Eight plates were exposed 45 cm. below and 45 cm. in front of the mouth of the speaker and eight others 15 cm. below and 15 cm. in front. In all these cases the speaking was loud, and vociferous, in English and German, and the period of exposure was five minutes. Finally four plates were exposed 15 cm. in front of and 15 cm. below the mouth of the speaker who orated and coughed for three minutes.

The plates were incubated for four days and subcultures were made from all suspicious colonies. All showed large colony counts (87 to 1,200). Of the first eight plates exposed to spray from speaking 45 cm. away, one only showed a single colony of the acid-forming streptococci. Of the eight plates exposed to spray from speaking 15 cm. in front of and 15 cm. below the mouth, three gave positive results. Two of the plates showed each a single colony, the third was wholly reddened so that the number of acid colonies could not be exactly determined. The streptococcus colonies were not in isolated pure cultures but in mixt colonies each of which was obviously derived from a droplet of saliva containing many forms. Of the 16 plates examined in these two series three were overgrown with an alkaline spreader which might have obscured any streptococci present.

Finally of the four plates exposed to the spray from coughing at a distance of only 15 cm. from the mouth, two were overgrown with alkaline spreaders and so may be excluded from consideration. Of the other two one showed two droplet colonies and the other four. All six on cultivation showed the presence of mouth streptococci.

Altogether it appears from these experiments with the mouth streptococci that normal mouth bacteria are much less easily discharged into the air than are artificial cultures introduced into the mouth. Loud speaking and even coughing did not deposit very large numbers of mouth streptococci even upon plates within 15 cm. of the mouth; and the quantitative examination of 74 liters of air exposed to the spray from loud and vigorous speaking at distances of 35 cm. to 2.4 meters from the mouth failed to show the presence of mouth streptococci in any instance.

SUMMARY AND CONCLUSIONS.

It appears from these experiments, as it appeared in the German investigations which have been reviewed above, that bacteria with which the mouth has been inoculated are discharged in the act of speaking in large numbers, and to considerable distances. Positive results were obtained as far as 7.5 meters from the speaker; and the average number of bacteria discharged over the whole surface of a room (6×8 meters) by 15 minutes' loud speaking was 646 per square meter. These results coincide closely with those obtained by Lascht-

schenko, Koeniger, and Gordon. A still more extensive spread of mouth spray would of course result from coughing and sneezing.

Most of the particles in the mouth spray are rather coarse and settle out rapidly. In fact the spray is like rain falling through the air, rather than mist suspended in it. Koeniger showed that 60 per cent of the bacteria in the mouth spray disappeared from the air in 10 minutes, while after 20 minutes less than 10 per cent of the original number remained. In estimating aerial infection proper, it is the bacteria suspended in the air which are important because these only could be inhaled with the inspired air. Our second object was to attain a quantitative measure of the extent of such aerial infection; and we found that after inoculating the mouth with a rich culture of *B. prodigiosus* and speaking loudly and with vigorous enunciation for 15 minutes only seven colonies of the specific germ could be obtained from 140 liters of air collected at the close of the speaking from various points in front of the speaker. The true aerial infection is therefore relatively small by comparison with the distribution of the heavier particles of spray.

Experiments with fresh cultures introduced in large amounts upon the surfaces of the mouth give an exaggerated idea of the quantitative importance of the mouth spray. We therefore attempted finally to control the investigation by a study of the distribution of the mouth streptococci which had been suggested by Gordon as offering a promising test of normal mouth pollution. Gordon easily detected these organisms by exposing plates after speaking for various lengths of time. We found them on plates by similar methods; but even at a distance of only 15 cm. from the mouth of a subject who coughed for three minutes the number of droplets containing mouth streptococci was small. Quantitative tests of the air itself from points 35 cm. to 2.4 meters in front of speakers who spoke vigorously in English or German failed to show any mouth streptococci in 74 liters of air examined.

It might be surmised that in pathological conditions bacteria may be more readily dislodged from the mucous membranes than would be the case under normal conditions. Thus the mouth streptococcus may furnish too low an index for measuring the mouth spray, as the *B. prodigiosus* furnishes too high a one. Experiments carried out with coughing consumptives by Laschtschenko and Heymann, how-

ever, bear out the conclusion that the actual extent of air infection is but small. Out of five air samples of 10 cu.m. each examined by Laschtschenko only two contained tubercle bacilli; out of seven samples of 1 cu.m. each, examined by Heymann, only two gave positive results.

It is not intended to minimize the real danger from mouth spray. Large numbers of bacilli are discharged in coughing by some consumptive patients. Ziesché found in one case 20,000 tubercle bacilli on a glass plate 324 sq.cm. in area exposed for half an hour from 40-80 cm. in front of the mouth of a phthisical patient. The infection by mouth spray of food or of other objects later brought directly or indirectly in contact with the mouth is a real and important danger. Flügge's practical recommendations are fully warranted by all the facts: "During strong paroxysms of coughing, the consumptive should keep at arm's length from his companions and should hold a handkerchief before his mouth. In workrooms, offices, and such places the space between the heads of the workers should be at least 1 meter."

On the other hand all this furnishes no basis for a belief that tuberculosis or any other disease is contracted to an appreciable extent through the inspired air. The mouth spray is a fairly coarse rain which quickly settles downward. Even in artificial experiments with *B. prodigiosus* the actual extent of air pollution, when quantitatively measured, appears to be small; and when infection by normal mouth streptococci or tubercle bacilli is studied it is much smaller still.

These conclusions are in harmony with the conviction now generally gaining ground that aerial infection of any sort is a minor factor in the spread of zymotic disease; and in regard to tuberculosis they accord well with the opinion that ingestion rather than inhalation is the principal channel of infection even in the case of bacilli originally discharged through the air in the form of mouth spray.

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